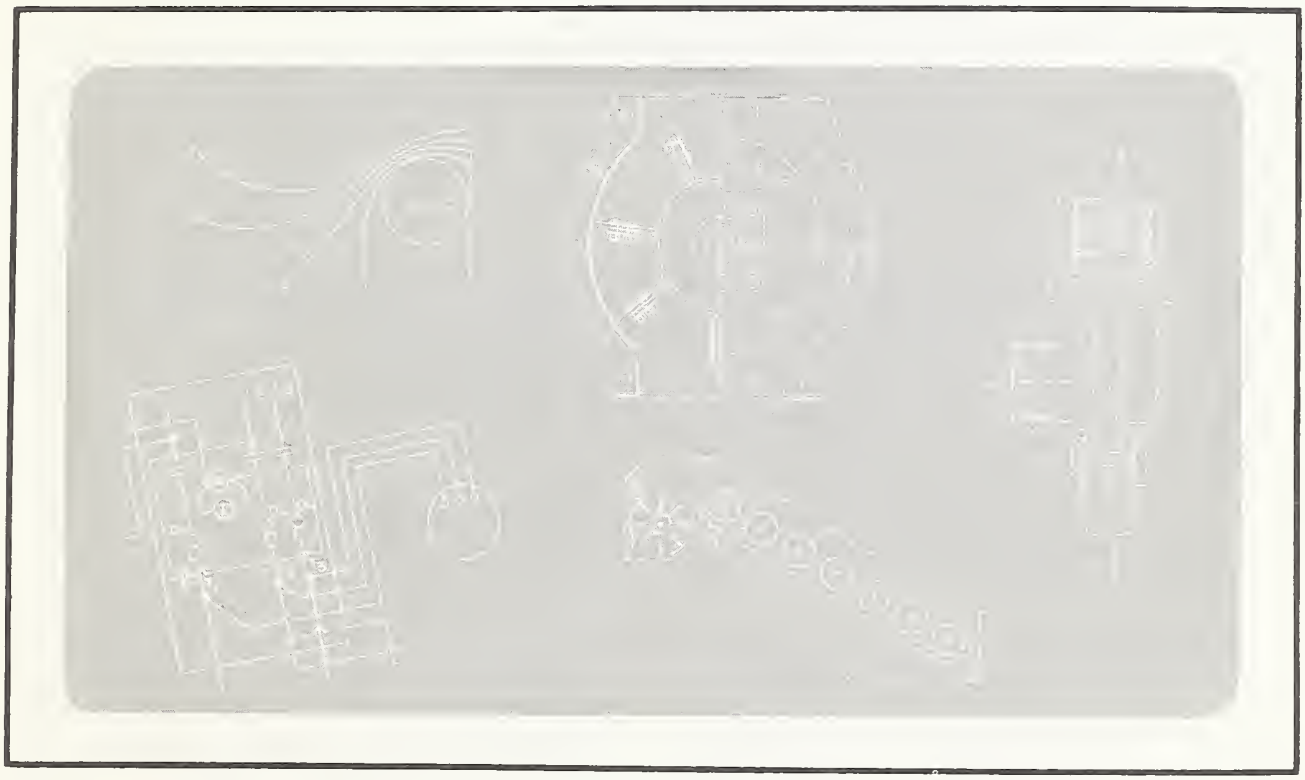
ISSN 0193-3728

Hand Inoculator for Dispensing Lepidopterous Larvae



U.S. Department of Agriculture
Science and Education Administration
Advances in Agricultural Technology • AAT-S-9/October 1979

CONTENTS

ILLUSTRATIONS

The authors thank John Mihm, entomologist, International Maize and Wheat Improvement Center, for sending us an inoculator. Margie Mertaugh, Boll Weevil Research Laboratory, drew the illustrations, and Frank Benci of the same laboratory provided figure 3.

This publication is available from the Crop Science and Engineering Research Laboratory, P.O. Box 5367, Mississippi State, Miss. 39762.

Science and Education Administration, Advances in Agricultural Technology, Southern Series, No. 9, October 1979.

Published by Agricultural Research (Southern Region), Science and Education Administration, U.S. Department of Agriculture, P.O. Box 53326, New Orleans, La. 70153.

Hand Inoculator for Dispensing Lepidopterous Larvae

By Frank M. Davis and Thomas G. Oswalt[1]

ABSTRACT

A hand-operated inoculator was designed and built to dispense a precise amount of corncob grits containing a premeasured number of southwestern corn borer, *Diatraea grandiosella* Dyar) larvae into diet-filled cups. This inoculator, which costs about $4 (1979 prices) plus labor to fabricate, eliminates the laborious task of transferring each larva with a small artist's brush to a diet-filled cup. Index terms: *Diatraea grandiosella* Dyar, inoculators, insect-rearing equipment, Lepidoptera.

INTRODUCTION

The U. S. Department of Agriculture greatly increased its research program for the southwestern corn borer, *Diatraea grandiosella* Dyar, in 1976. This required the rearing laboratory at Mississippi State, Miss., to increase its capacity several fold in order to satisfy the additional requirements for insects to be used in plant-resistance studies. Before 1976, the rearing techniques for the southwestern corn borer were those commonly used in small rearing programs where the work was accomplished by hand procedures (Davis 1976). When the rearing program increased from small to medium size, hand procedures became too inefficient and costly. Thus, efforts were begun to automate the various rearing procedures. Development of an efficient automated diet dispenser was the first step (Davis et al. 1978). The second step toward automation was to develop a procedure for infesting diet-filled cups with eggs or larvae and thus eliminate the very laborious method of transferring each first-instar larva with a small brush to each diet-filled cup.

A group of entomologists (John Mihm, Frank Peairs, and Alejandro Ortega) at the International Maize and Wheat Improvement Center at El Batan, Mexico, developed a hand-operated larval dispenser (that they refer to as the "bazooka") to infest corn plants with a premeasured number of lepidopterous larvae mixed in corncob grits (CIMMYT Review 1978). Our objective was to design and develop an inoculator (utilizing the bazooka's principle of granular-material transfer from a supply source to an exit tube) that would dispense southwestern corn borer larvae for infesting diet-filled cups in the laboratory. This report describes the resulting inoculator, which is relatively simple to construct, inexpensive, easy and fast to operate, and accurate.

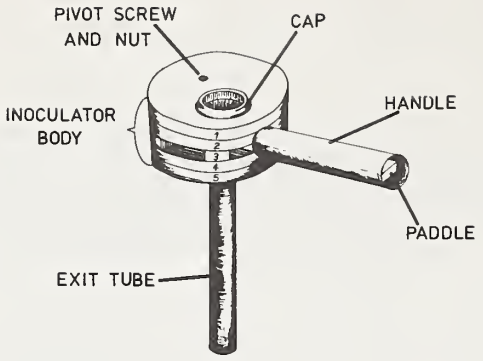
MATERIALS, CONSTRUCTION, AND COST

Fabrication of the inoculator requires one each of the following materials:

Plexiglas, 12- by 12- by ¼-inch.
Pipe, CPVC[2] (hot), ½-inch (i.d.), 6-inch-long.
Pipe, PVC, ⅝-inch (i.d.), 4-inch-long.
Screw (and nut), stainless-steel, ⅛- by 1-inch.
Bottle cap, polyethylene, 28-millimeter-diameter.

[1] Research entomologist and biological technician, Crop Science and Engineering Research Laboratory, Science and Education Administration, U.S. Department of Agriculture, P.O. Box 5367, Mississippi State, Miss. 39762.

[2] CPVC—"C" is the type of plastic used in making the polyvinyl chloride (PVC) pipe.



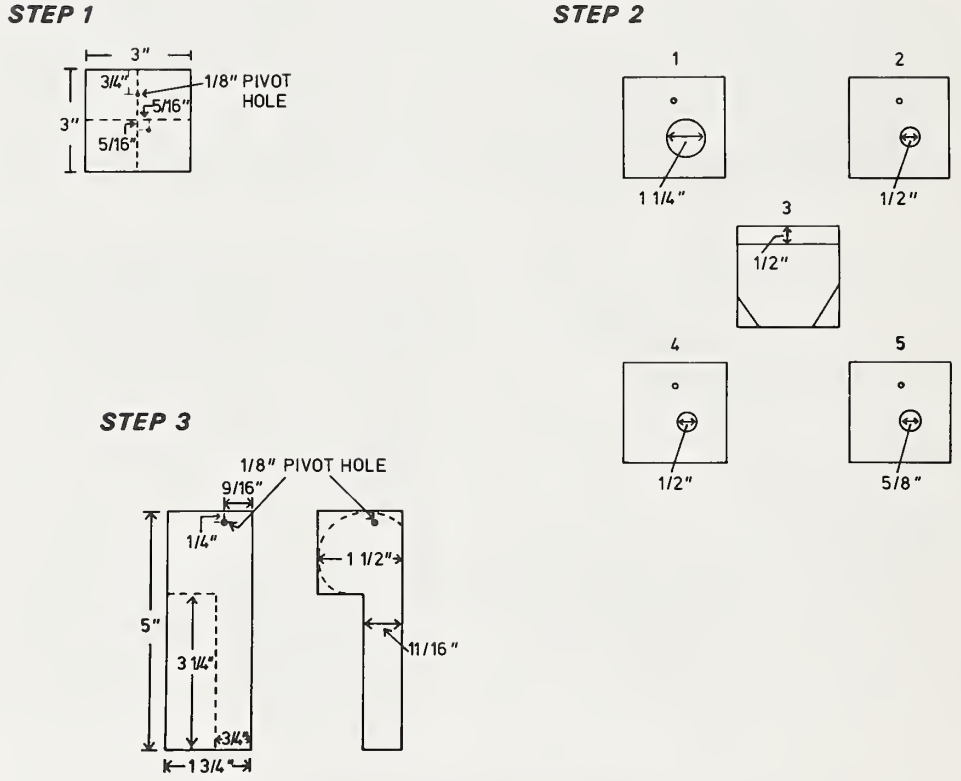


FIGURE 1.—Constructing the inoculator, steps 1 through 3.

The design of the inoculator and the construction steps, which are given below, are illustrated in figures 1 and 2.

Steps 1 and 2.—Cut out five 3-inch squares of ¼-inch-thick Plexiglas. Drill the different-size holes with Plexiglas drill bits (obtained from Norrell, Inc., Memphis, Tenn.) at the designated positions in layers 1, 2, 4, and 5. Cut layer 3 as shown; the pieces serve as a spacer and as stops for the paddle and do not require holes.

Step 3.—Cut out "paddle" from 1¾- by 5-inch piece of ¼-inch-thick Plexiglas. Drill ⅝-inch hole in head of paddle. Using a belt or disk sander, round off paddle head, leaving at least 1½ inches across the head.

Step 4.—Position layer 2 on the 3- by ½-inch section of layer 3, with its ½-inch-diameter hole on the left side of center. Position layer 4 against underside of layer 3, with its ½-inch-diameter hole on the right side of center. Place paddle into the space between layers 2 and 4 and insert pivot screw, making sure that head of the paddle is at

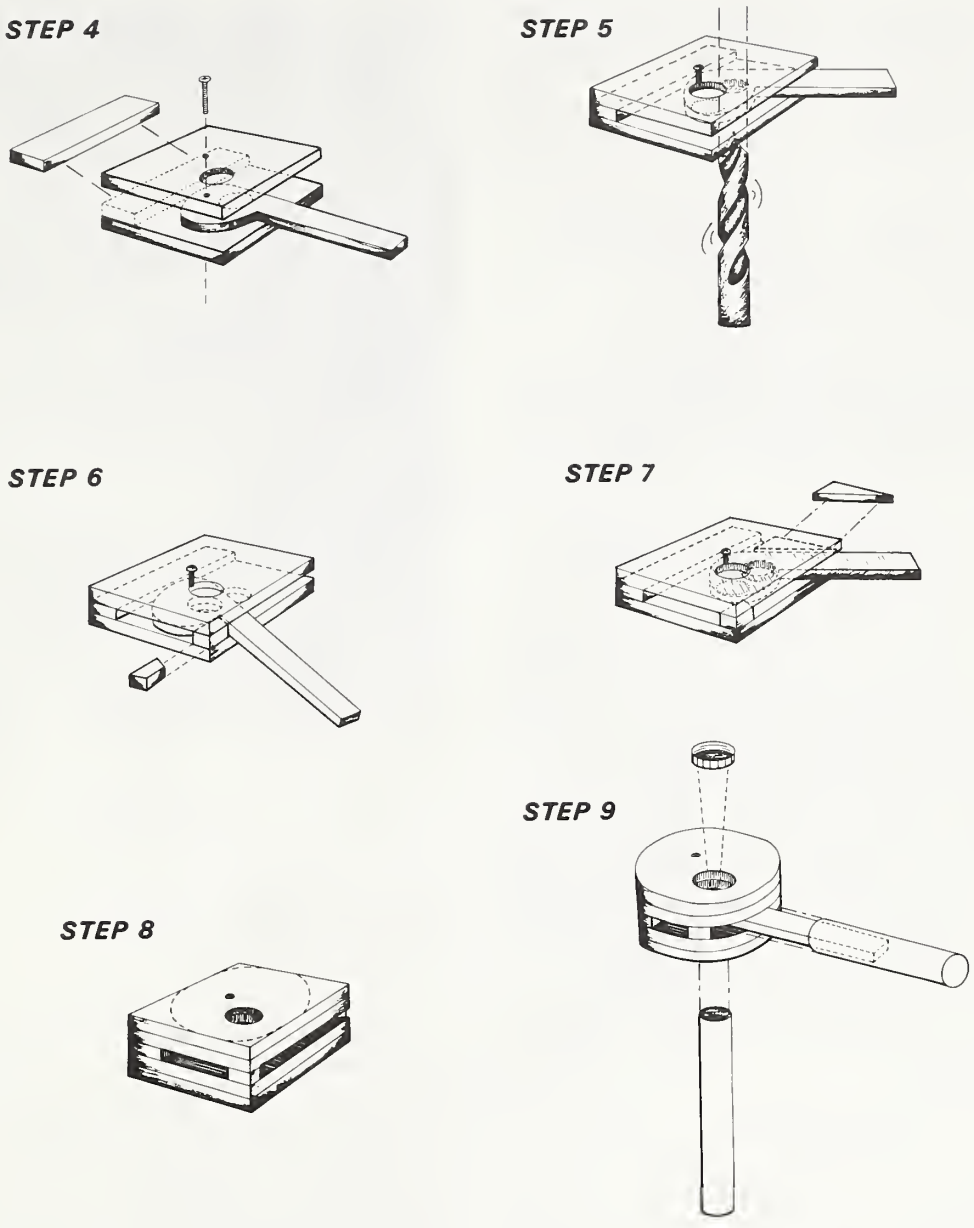


FIGURE 2.—Constructing the inoculator, steps 4 through 9.

least one-fourth of an inch from this section of layer 3. Remove pivot screw and paddle, and cement layers 2, 3, and 4 together, using methylene chloride applied with a large syringe and needle. Prevent methylene chloride from running into the area where the paddle is to move back and forth.

Step 5.—Place paddle into the space and insert pivot screw. Move paddle to the right until the paddle head covers the ½-inch-diameter hole in layer 2. Insert ½-inch-diameter drill bit through the ½-inch-diameter hole in layer 4 and start paddle hole. Remove pivot screw and paddle and complete ½-inch hole at the place marked by the drill bit.

Step 6.—Place paddle into the space and insert pivot screw. Move paddle to the point where its ½-inch-diameter hole matches exactly with the ½-inch-diameter hole in layer 2. Insert stop (cut from layer 3) so that the holes match each time the paddle is in this position. Remove pivot screw and paddle, and cement stop in place.

Step 7. — Place paddle in space and insert pivot screw. Move paddle to the point where its ½-inch-diameter hole matches exactly with the ½-inch-diameter hole in layer 4. Remove pivot screw and paddle, and cement stop (cut from layer 3) in place.

Step 8. — Position layer 1 onto layer 2 so that its 1¼-inch-diameter hole is directly over the ½-inch-diameter hole in layer 2. Position layer 5 so that its ⅝-inch-diameter hole is directly over the ½-inch-diameter hole in layer 4. Cement layers 1 and 5 onto layers 2 and 4, respectively. Using a belt or disk sander, grind inoculator body (after allowing to dry completely) to an elliptical shape that fits comfortably between thumb and forefinger.

Step 9. — Drill center out of polyethylene bottle cap. Insert cap into 1¼-inch-diameter hole in layer 1, and secure with hot-melt glue applied by an electric glue gun (USM Corporation, Consumer Products Division, Reading, Pa.). Countersink pivot holes in layers 1 and 5. Insert pivot screw and moderately tighten nut on it. Insert 6-inch CPVC (½-inch-i.d.) pipe into the ⅝-inch-diameter hole in layer 5 and secure with methylene chloride. Place 4-inch PVC (⅝-inch-id.) pipe over the paddle handle. Fill in the space between paddle handle and PVC pipe with silicone calking material. Paint inoculator with enamel spray paint. Avoid getting paint on paddle head or inside the space where the paddle moves.

The cost of materials for fabricating this inoculator is $4 (1979 prices). About 6 hours of labor are required.

PROCEDURES

Sterilization of Corncob Grits

It is important that the corncob grits be sterilized to avoid microbial contamination of the diet. Our procedure for sterilizing and storing the corncob grits (in 10-pound lots) is as follows: (1) sterilize in steam autoclave at 121° C (250° F) for 2 hours, (2) place in oven for 30 minutes at 120° C (248° F) to remove moisture, (3) cool and then add 1.36 grams of the fungicide folpet[3] and mix thoroughly by rolling in a container on a laboratory jar mill for 2 hours, and (4) store in 1-quart plastic Ziploc bags until needed.

[3] *N*-[(trichloromethyl)thio]phthalimide.

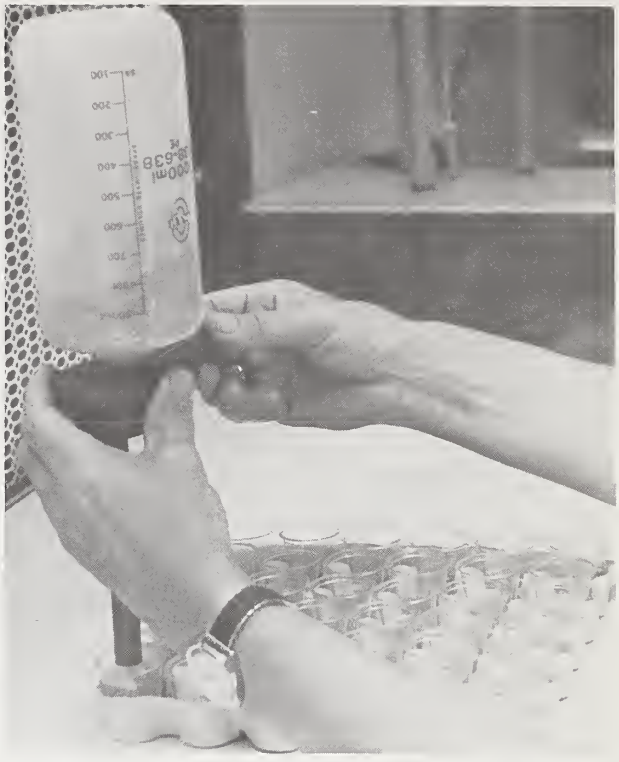


FIGURE 3. — Using the inoculator to infest diet-filled cups with larvae.

Measurement of Larvae for Delivery

The eggs, which are oviposited on wax-paper sheets, are allowed to hatch in 1-gallon jars. A small amount of corncob grits is poured into each jar, and the paper sheets are removed; the larvae easily mix with the corncob grits. The mixture is poured into a supply bottle (1,000-milliliter polyethylene wash bottle with 28-millimeter cap). Additional corncob grits are added to the mixture until the desired number of larvae per delivery is obtained. Occasional gentle tumbling of the mixture insures uniform larval distribution.

Operation of Inoculator

The inoculator functions by moving the paddle from stop to stop. A precise amount of corncob grits moves from the supply bottle (fig. 3) into the ½-inch-diameter hole in the paddle. When the paddle is operated, the grits move to the exit hole and down through the exit tube into the diet-filled cups. Since the paddle hole governs the amount of

each delivery, smaller amounts per delivery can be attained by simply making the paddle hole smaller. The size of corncob grits that works best in the inoculator is # 2040 (obtained from The Andersons, Maumee, Ohio). The inoculator can be used to infest cups as shown in figure 3, or it can be fastened to a laboratory ring stand, with the cups placed under the exit tube. When the dispensing is completed, the inoculator is sterilized in 10-percent Chlorox solution.

Our inoculator has proven to be an efficient method of infesting diet-filled cups with southwestern corn borer larvae, and using it is at least twice as fast as the old method of transferring each larva by brush. We feel that this inoculator could be used in many lepidopterous rearing programs.

REFERENCES

CIMMYT Review.
1978. International Maize and Wheat Improvement Center, Mexico City.

Davis, F. M.
1976. Production and handling of eggs of the southwestern corn borer for host plant resistance studies. Miss. Agric. For. Exp. Stn. Tech. Bull. 74, 11 pp.

Davis, F. M; Oswalt, T. G.; and Boykin, J. C.
1978. Insect diet dispenser for medium-size rearing programs. U.S. Agric. Res. Serv. [Rep.] ARS-S-182, 3 pp.

U.S. DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION
P. O. BOX 53326
NEW ORLEANS, LOUISIANA 70153

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300

POSTAGE AND FEES PAID
U. S. DEPARTMENT OF
AGRICULTURE
AGR 101

FIRST CLASS

☆U.S. GOVERNMENT PRINTING OFFICE:1979-671-037/11A